THE FERMENTATION OF ARABINOSE AND XYLOSE BY CERTAIN AEROBIC BACTERIA¹

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During a study of the aerobic flora from lake water, silage and vinegar, it was found that many of these organisms attack pentose sugars. In contrast to the acid formers which are described in earlier papers (Fred et al, 1919; 1921), these aerobic organisms ferment the pentose sugars, arabinose and xylose more slowly and produce only a small amount of acid. The total yield of fixed acid is far too small to account for the carbohydrate consumed. Products other than acids must be formed and various experiments were carried out in order to study the products of the aerobic fermentation and the proportion in which they are found.

Six cultures of bacteria representing various groups of the more common aerobic types were used in this study. *Bacillus vulgatus* (Flügge) Trevisan, synonymy *Bacillus mesentericus vulgatus*, was isolated from the mud of Lake Mendota; *Acetobacter sorbose* and *Acetobacter xylinum* were obtained from the American Museum of Natural History, and originally came from Bertrand; while three chromogenic forms, one a rod which answers to the description of *Bacterium herbicola aureum* (Düggeli 1904) and the others two coccus forms designated "Organism A, and B" were isolated from green sweet corn.

Some of the characteristics of these aerobic pentose fermenters are shown in table 1. Aside from the great difference in morphology, these forms are readily distinguishable by definite

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TABLE 1
The characteristics of certain aerobic pentose fermenters

NUMBER	BACILLUS VULGATUS	ACETOBACTER SORBOSE	ACETOBACTER XYLINUM	ORGANISM A	ORGANISM B	BACTERIUM HERBICOLA AUREUM
1. Form.....	Rod	Rod	Rod	Coccus	Coccus	Rod
2. Spores.....	Present	None	None	None	None	None
3. Flagella....	Present	Present	Present	None	None	Present
4. Pigment.....	Fawn-colored	Light-yellow	Brownish-yellow	Orange	Yellow	Light-yellow
5. Gram stain.....	Positive	Positive	Positive	Positive	Positive	Negative
6. Catalase test.....	Very strong	Strong	Fair	Strong	Very strong	Very strong
7. Carbinol test						
Glucose.....	Strong	Fair	Medium	None	None	None
Arabinose.....	Strong	Trace	Medium			
Xylose.....	None	None	None			
8. Gelatin.....	Liquefied	Not liquefied	Not liquefied	Slow liquefaction	Rapid liquefaction	Not liquefied
9. Litmus milk.....	Reduced, soft curd later digestion	Reduced, brown serum zone, soft curd	Reduced, pink serum zone, soft curd	Alkaline, reduced	Alkaline, digestion	Yellow, no change

physiological characters. These differences are perhaps best seen from a comparison of the action of the bacteria in milk and in gelatin. On solid and liquid media, *B. vulgatus* forms a wrinkled membrane which spreads rapidly over the entire surface. *Acetobacter sorbose* on glucose yeast-water agar forms a pale yellow film. In liquid the medium becomes turbid throughout. *Acetobacter xylinum* forms a thick pink to brown membrane on glucose yeast-water agar slopes. The coccus forms A and B produce a raised profuse growth on agar slopes and a decided turbidity in liquid media.

In yeast water solutions the three chromogenic forms ferment glucose, mannose, fructose, sucrose, maltose, lactose, melezitose salicin, mannitol and glycerol with the production of a small amount of fixed acid. Raffinose, alpha-methyl glucoside, dulcitol, inulin and starch are not attacked. Aside from total acidity, the amount of sugar fermented was also determined. In general it was found that *B. herbicola aureum* is the most active fermenter of the three, destroying from 50 to 60 per cent of the sugar in the first ten days. Organism B in turn is more active in the fermentation of sugars than Organism A.

THE FERMENTATION OF ARABINOSE AND XYLOSE BY *B. VULGATUS* *ACETOBACTER SORBOSE* AND *ACETOBACTER XYLINUM*

In preliminary experiments to determine the fermentation of the pentoses by these organisms 25 cc. cultures of 2 per cent sugar medium were grown in 100 cc. Erlenmeyer flasks. The source of the nitrogen was varied. However, no well defined difference in fermentation was noted from the use of beef broth or of peptone broth in place of the yeast water. In general, the yeast water proved slightly superior and hence in all of the quantitative work which follows, this medium was employed.

The methods for measuring the products of fermentation with these aerobic forms were essentially different from those used in the work on acid fermentation of pentoses by *Lactobacilli*. The fermentation was carried out in a closed system, figure 1, through which carbon dioxide-free air was drawn. The fermentation flask *C* was closed with a two-hole rubber stopper, which

fitted tightly in the neck of the flask (see *F*). Through one hole was passed a long glass tube reaching almost to the surface of the culture solution. The other end of this glass tube carried a cotton plug *A*. Through the second hole in the rubber stopper a short glass tube *B* was passed. The upper end of this tube *B* was closed by means of a piece of rubber tubing, a screw

FIG. 1. FLASK FOR THE GROWTH OF AEROBIC BACTERIA AND THE ABSORPTION OF EVOLVED CARBON DIOXIDE

pinch-cock, and a cotton plug, protected by a short inverted test tube. Through tube *B*, samples were removed for analysis. The flask *C* was connected to a 50 cc. pipette, which in turn passed into the alkali flask *E*. This flask contained a solution of 30 per cent KOH. By means of a bottle aspirator, a slow current of CO₂-free air, was drawn through the system of flasks to remove evolved gases. The carbon dioxide was determined

by double titration according to the method of Scales (1920). As a check on the Scales method CO_2 was also determined by the gasometric method of Van Slyke (1917).

The cultures were connected in series and aspirated continuously with a current of CO_2 -free air. During the incubation period, usually four weeks, the entire apparatus was kept at 28°C . At irregular intervals the CO_2 receivers were removed and the CO_2 determined. After some preliminary tests, it was found that this kind of fermentation flask if carefully set up may be kept for months without contamination and without any loss of CO_2 . No attempt was made to measure gaseous products other than CO_2 .

Since all of the organisms showed an appreciable growth in the yeast water alone, each organism was grown in this medium and the carbon dioxide found was deducted from the total gas production on the yeast water plus sugar. All three cultures, *B. vulgatus*, *A. sorbose* and *A. xylinum* break down xylose with the production of large amounts of CO_2 . This substance is far in excess of all others and accounts for a large part of the sugar consumed. The data obtained from the fermentation of xylose in yeast water are given in table 2. As compared with the acid fermentation of xylose by *Lactobacilli*, the most noticeable difference is the large CO_2 production and the absence of acids. Since atmospheric oxygen is utilized in the fermentation process, a direct comparison cannot be made between the total weight of products formed and the weight of sugar consumed.

Gruner and Fraser (1921) in their study of the *Bacillus mesentericus* group and allied organisms, called attention to the action of this group on sugar media. They found that acid formation from glucose usually reaches a maximum by the second or third day and on longer periods of incubation fails to increase. Results somewhat similar to those of Gruner and Fraser were obtained with *B. vulgatus*. At first there was a small amount of acid, which in the older cultures disappeared almost completely.

Of special interest is the production of acetone and ethyl alcohol from xylose by both *B. vulgatus* and *A. xylinum*. The acetone organisms which have been isolated and studied

B. macerans (Schardinger, 1905), *B. granulobacter pectinovorum* (Speakman, 1920), *B. acetoethylicum* (Northrop, Ashe and Senior, 1919), *B. butylicus* (Ricard, 1919) are facultative anaerobic rod forms. In contrast to the organisms described in this paper these acetone forming bacteria produce acid, usually acetic or butyric, together with the neutral substances, acetone and alcohol. A recent paper by Berthelot and Ossart (1921) gives the results of acetone production by 217 cultures of bacteria isolated from soil, water and manure. Their data are substantially in agreement with the figures of table 2.

TABLE 2
Total fermentation products from xylose

Xylose at beginning 1.89 per cent. Calculated for 100 cc. of culture.

PRODUCTS	B. VULGATUS	A. SORBOSE	A. XYLINUM
	gm.	gm.	gm.
Carbon dioxide			
From xylose and yeast water.....	2.3990	2.1702	2.2223
Yeast water alone.....	0.8068	0.3457	0.8437
From xylose.....	1.3922	1.8245	1.3786
Ethyl alcohol.....	0.0784	0.0	0.3698
Acetone.....	0.0088	0.0	0.1847
Total products.....	1.4794	1.8245	1.9331
Xylose fermented.....	1.4007	1.6090	1.2530

The two organisms *A. sorbose* and *A. xylinum*, behaved somewhat differently from the description given by Brown (1886) and by Bertrand (1904). The general cultural characteristics answer to those given in the original description, but the products of fermentation were markedly different. Instead of forming arabonic acid from arabinose and xylonic acid from xylose, as described by the original investigators, the main products of fermentation were found to be carbon dioxide by *A. sorbose* and carbon dioxide, ethyl alcohol and acetone by *A. xylinum*. The formation of these two neutral products was totally unexpected and to be sure that it was not due to contamination, this experiment was repeated several times and always with the same results. *A. xylinum* produces acetone and alcohol from xylose in

large amounts. If calculated in percentage of the sugar consumed, the acetone is equivalent to 10.8 per cent, the alcohol to 18.8 per cent. These values are of special interest for comparison with those recorded in papers by Speakman (1920) for *B. granulobacter pectinovorum* and by Artzberger et. al (1920) for the acetone organism, *B. acetoethylicum*. The percentages are approximately of the same order as those reported by these investigators.

EFFECT OF AGE ON ACETONE PRODUCTION

In preliminary tests it was found that the age of the culture influences the amount of acetone. The next experiment was

TABLE 3
Effect of age of cultures on acetone and alcohol production
Calculated for 100 cc. of culture.

PRODUCT	AGE OF CULTURE	B. VULGATUS	A. XYLINUM
	days	gm.	gm.
Acetone.....	6	0.0902	0.0651
Acetone.....	20	0.0024	0.0843
Ethyl alcohol.....	6	0.1122	0.1325
Ethyl alcohol.....	20	0.0147	0.1472
Xylose fermented in twenty days		90.26 per cent	70.92 per cent

undertaken in an attempt to find out the amount of acetone at different ages of the cultures, *B. vulgatus* and *A. xylinum*. Table 3 shows the values obtained for acetone and alcohol at varying ages. These two cultures are strikingly different. *B. vulgatus* forms large amounts of acetone and alcohol in young cultures and later shows a decided decrease in these two substances, while *A. xylinum* shows only a small amount of acetone and alcohol in young cultures but increases continually to a large amount in old cultures.

THE FERMENTATION OF ARABINOSE AND XYLOSE BY *B. HERBICOLA*
AUREUM AND ORGANISMS A AND B

Because of the frequent occurrence of these three chromogenic types on green plants, their action on the pentose sugars is of particular importance. Although widely distributed in nature and easily isolated, these three forms fail to exhibit any striking physiological characteristics. No doubt these, or closely related organisms, inhabitants of living plant tissue, play an important rôle in nature.

TABLE 4
Total fermentation products from pentoses
Calculated for 100 cc. of culture.

PRODUCTS	XYLOSE AT BEGINNING 1.9765 PER CENT			ARABINOSE AT BEGINNING 1.8970 PER CENT
	Organism A	Organism B	<i>B. herbicola</i> aureum	<i>B. herbicola</i> aureum
	gm.	gm.	gm.	gm.
Carbon dioxide				
From sugar and yeast water.....	0.5810	0.6800	1.6667	2.3257
From yeast water alone.....			0.3946	0.3946
From sugar.....			1.2721	1.9311
Sugar fermented.....	0.6900	0.6850	1.5654	1.6328

The apparatus used and methods of study described in the first part of this paper were followed. After thirty days the cultures were analyzed and the results shown in table 4. These two coccus forms are slow fermenters of xylose destroying about 30 per cent, but *B. herbicola aureum* attacks the pentose sugars much more vigorously. In thirty days about 79 per cent of the xylose and 81 per cent of the arabinose was fermented. The products of fermentation are almost entirely CO₂ and a small amount of acid. From *B. vulgatus* 0.076 gram of non-volatile acid calculated as lactic was obtained for 100 cc. of medium.

SUMMARY

This report deals with the fermentation of the pentose sugars by certain aerobic bacteria, viz., *B. vulgatus*, *A. sorbose*, *A. xylinum*, *B. herbicola aureum* and two yellow coccus forms designated Organism A and B. The rate of fermentation of xylose, and in certain cases arabinose, by these aerobic forms is much slower than is noted with the facultative anaerobic bacteria.

The products of the fermentation of the pentose sugars depends upon the kind of organism and the ratio of the products on the age of the culture. *B. vulgatus* ferments xylose with the production of acetone, ethyl alcohol, carbon dioxide and a small amount of fixed acids. As the culture becomes older the ratio of acetone and ethyl alcohol to carbon dioxide decreases. The maximum amount of acetone and alcohol is found about the sixth day after inoculation.

In the case of *A. xylinum* an increase in the age of the culture is accompanied by an increase in the yield of acetone and alcohol. Although in different proportions the substances obtained in the breaking down of xylose, *A. xylinum* are the same as noted with *B. vulgatus*. *A. sorbose*, *B. herbicola aureum* and the two coccus forms ferment the pentoses slowly and the chief end product is carbon dioxide and a trace of acid.

It may be concluded that in nature many of the commonly occurring aerobic forms of bacteria attack the pentose sugars and form as the chief end products, neutral substances such as acetone and ethyl alcohol, and carbon dioxide.

An apparatus for conducting the fermentations under aerobic conditions is described.

REFERENCES

- ARZBERGER, C. F., PETERSON, W. H., AND FRED, E. B. 1920 Jour. Biol. Chem., **44**, 465.
BERTHELOT, A., AND OSSART, E. 1921 Compt. Rend., **173**, 792.
BERTRAND, G. 1904 Ann. de Chimie et de Physique, 81 Ser., **3**, 181.
BROWN, A. J. 1886 Chem. Soc. Jour. (London) (Trans.), **49**, 432.
DÜGGELI, MAX 1904 Centr. Bakt. 2te Abt., **13**, 56, 198.
FRED, E. B., PETERSON, W. H., AND ANDERSON, J. A. 1921 Jour. Biol. Chem., **48**, 385.

- FRED, E. B., PETERSON, W. H., AND DAVENPORT, A. 1919 Jour. Biol. Chem. **39**, 347.
- GRUNER, O. C., AND FRASER, J. R. 1921 Jour. Inf. Dis., **10**, 210.
- NORTHROP, J. H., ASHE, L. H., AND SENIOR, J. H. 1919 Jour. Biol. Chem., **39**, 1.
- RICARD, E. 1919 Jour. Soc. Chem. Ind., (London), **38**, 787.
- SCALES, F. M. 1920 Science, n.s., **51**, 214.
- SCHARDINGER, F. 1905 Centr. Bakt., 2te., Abt. **14**, 772.
- SPEAKMAN, H. B. 1920 Jour. Biol. Chem., **41**, 319.
- VAN SLYKE, D. D. 1917 Jour. Biol. Chem., **30**, 347.